Single-cell western blotting.

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Public Summary:
Heterogeneity is inherent to cellular processes, including differentiation. Western blotting is a sensitive method for detecting and quantifying specific proteins within a mixed sample. Here we report on a single cell western blotting method capable of measuring, with high specificity, cell-to-cell protein heterogeneity within complex populations of cells. This method permits simultaneous assays of approximately 2000 individual cells in less than four hours. We applied the method to study immature neural stem cells and their differentiation over a six day period under conditions that yielded both astroctyes and neurons. The method successfully reported the presence of proteins associated with neural differentiation and identified a variant of the protein nestin that was sharply downregulated with development. This single cell Western blotting method overcomes limitations of antibody fidelity and sensitivity in other single-cell protein analysis methods and constitutes a versatile tool for the study of complex cell populations at single-cell resolution.

Scientific Abstract:
To measure cell-to-cell variation in protein-mediated functions, we developed an approach to conduct approximately 10(3) concurrent single-cell western blots (scWesterns) in approximately 4 h. A microscope slide supporting a 30-mum-thick photoactive polyacrylamide gel enables western blotting: settling of single cells into microwells, lysis in situ, gel electrophoresis, photoinitiated blotting to immobilize proteins and antibody probing. We applied this scWestern method to monitor single-cell differentiation of rat neural stem cells and responses to mitogen stimulation. The scWestern quantified target proteins even with off-target antibody binding, multiplexed to 11 protein targets per single cell with detection thresholds of <30,000 molecules, and supported analyses of low starting cell numbers (approximately 200) when integrated with FACS. The scWestern overcomes limitations of antibody fidelity and sensitivity in other single-cell protein analysis methods and constitutes a versatile tool for the study of complex cell populations at single-cell resolution.